

THE TRANSIENT-ZONE IN THE HUMAN AND MOUSE ADRENAL GLAND¹

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ABSTRACT

The use of histological criteria to classify the stages in development of the transient-zone in the human and mouse adrenal gland may elucidate the question of structural and functional homology. The transient-zone differs in its development and probable function in these two mammalian species in three ways.

(1) With respect to time of appearance and disappearance, the transient-zone develops in man during the fourth week of gestation (Stage 1) and disappears from 28 weeks gestation to one year postnatal (Stage 4). In the mouse the transient-zone appears during the eight postnatal day (Stage 1) and disappears in the male at the onset of puberty and in the female during the first pregnancy (Stage 4).

(2) With respect to PAS-positive material, in 18-week-old human fetuses (Stage 3), juxtanuclear PAS-positive coarse granules surrounding a homogeneous cytoplasmic mass may represent the Golgi region. The presence of this material is not demonstrated in the mouse.

(3) With respect to lipid and cholesterol, a positive but slight reaction for lipid and a questionable reaction for the presence of cholesterol is seen in the transient-zone of man. In the mouse a positive reaction for lipid and cholesterol was never observed.

INTRODUCTION

In the adrenal cortex of the human fetus, Starkłówna and Węgrzynowski (1910) were the first to describe a special region which they named the "mark zone". Howard-Miller (1927) observed a zone of cells within the adrenal cortex of the newborn mouse which she termed the "x-zone", and which she considered to be functionally homologous and histologically comparable to the mark zone in man. Since that time, much work has been devoted to describing the development and function of this zone in man and other mammalian species (Benner, 1940; Crowder, 1957; Deanesly, 1928; Elliott and Armour, 1911; Gruenwald, 1946; Lanman, 1960, 1961; McIntosh, 1960; Howard-Miller, 1927-1928; Sucheston and Cannon, 1968b; Whitehead, 1932-1933).

The purpose of the present study was to compare the histological characteristics of the developing transient-zone (Sucheston and Cannon, 1968a) in the human and mouse adrenal gland. It was considered that use of histological criteria to classify the developmental stages of the transient-zone might elucidate its structural and functional homologies.

MATERIALS AND METHODS

Human adrenal glands from 50 autopsy specimens, ranging in age from one month gestation to 15 years, were obtained for histological examination. The age, sex, and cause of death of each specimen were obtained from hospital records.

Four Swiss albino mice (*Mus musculus*) (two males and two females) were sacrificed each day, from birth through the 30th day, by intraperitoneal injection of one-half ml of veterinary grade sodium nembutal (60 mg/ml) per kg of body weight. Four mice were subsequently sacrificed at two-day intervals for an additional 22 days.

The adrenal glands were fixed in formal-sublimate-acetic acid (FSA) (Movat, 1955) and in 10% neutral formalin. Paraffin sections cut at 4 μ were stained with hematoxylin and eosin and azan (Gurr, 1953). The alcian-blue periodic acid-Schiff reaction (AB/PAS) for glycoprotein (Mowry, 1956) was also performed on

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paraffin-sectioned material. Frozen sectioned material was utilized for the histochemical demonstration of total lipids (Chiffelle and Putt, 1951) and of cholesterol and its esters (Weber, Phillips, and Bell, Jr., 1956).

OBSERVATIONS

Stages in development of the transient-zone in man and the mouse were established by microscopic examination (Table 1). These are described below.

Human

Stage 1.—Four through eight weeks gestation.

Primary cortical proliferation began by the fourth week of gestation from coelomic epithelial cells between the root of the dorsal mesentery and the urogenital ridge. This disorganized mass of coelomic epithelium was composed of large, polyhedral cells with granular basophilic cytoplasm and indistinct cell boundaries. These cells composed the transient-zone (fig. 1a) and were negative to PAS, lipid, and cholesterol reactions.

Stage 2.—Eight and one-half weeks through seventeen weeks gestation.

The permanent cortex (fig. 1b) developed as a secondary proliferation of unorganized coelomic epithelial cells, which formed a cap over the transient-zone except on its medial aspect. The cells of the transient-zone remained large and polyhedral, but the granular cytoplasm was now acidophilic and cell boundaries were distinct; these cells were negative to PAS, lipid, and cholesterol reactions.

Stage 3.—Eighteen through twenty-seven weeks gestation.

The cells of the transient-zone now occupied approximately three-fourths of the entire adrenal gland and were characterized by juxtannuclear PAS-positive coarse granules surrounding a homogeneous cytoplasmic mass (fig. 3). The cells also showed the definite presence of lipid material and the possible presence of cholesterol.

Stage 4.—Twenty-eight weeks gestation through six months to one year postnatal. By the end of the first year, all that remained of the transient-zone were scattered cells adjacent to the medulla. These cells were PAS-positive and lipid-positive, and indicated a possible presence of cholesterol. At 36 weeks postnatal, a connective tissue stroma, present for approximately one week, formed an inner capsule separating the cortex from the medulla.

Stage 5.—Two years to puberty (11 to 15 years).

A definitive zonular pattern in the adrenal gland of both the male and female was established by: 1) proliferation of the permanent cortex to form the zona glomerulosa and outermost portion of the zona fasciculata; 2) maturation of the transient-zone to form the innermost portion of the zona fasciculata and the entire zona reticularis; and 3) growth of the medulla. No remnants of transient-zone cells were apparent.

Mouse

Stage 1.—Eight through sixteen days postnatal.

The permanent cortex was present at birth, while the transient-zone appeared gradually as juxtamedullary scattered clusters of cells by the second postnatal week (fig. 2a). These cells were small and compact, possessed deeply acidophilic cytoplasm and indistinct cell boundaries, and were negative to PAS, lipid, and cholesterol reactions.

Stage 2.—In the mouse the permanent cortex was present prior to the appearance of the cells of the transient-zone; therefore, Stage 2 was not comparable in man and the mouse.

Stage 3.—Seventeen through twenty-eight days postnatal.

The cells of the transient-zone now occupied approximately one-third of the entire adrenal gland (fig. 2b). These cells showed a gradation in cytoplasmic acidophilia from light acidophilia adjacent to the developing zona fasciculata to deep acidophilia in cells adjacent to the developing medulla. Moreover, the cells possessed no PAS-positive material and were negative for lipid and cholesterol.

Stage 4.—Twenty-nine through forty-five days postnatal.

By the time of sexual maturity in the male mouse, at approximately 45 days, the majority of cells of the transient-zone had disappeared. In the female mouse the cells of the transient-zone were present until the first pregnancy. The cells of the transient-zone remained negative for PAS, lipid, and cholesterol reactions in both sexes. With the loss of cytoplasmic acidophilia in the cells of the transient-zone, a permanent inner connective-tissue capsule (fig. 4) separating the cortex from the medulla became evident.

Stage 5.—Forty-six days through fifty-two days postnatal.

A definitive zonular pattern in the adrenal gland of both the male and the female mouse was established by what appeared to be the same processes as those occurring in man (State 5). A band of cells adjacent to the inner connective-tissue capsule may represent remnants of the transient-zone.

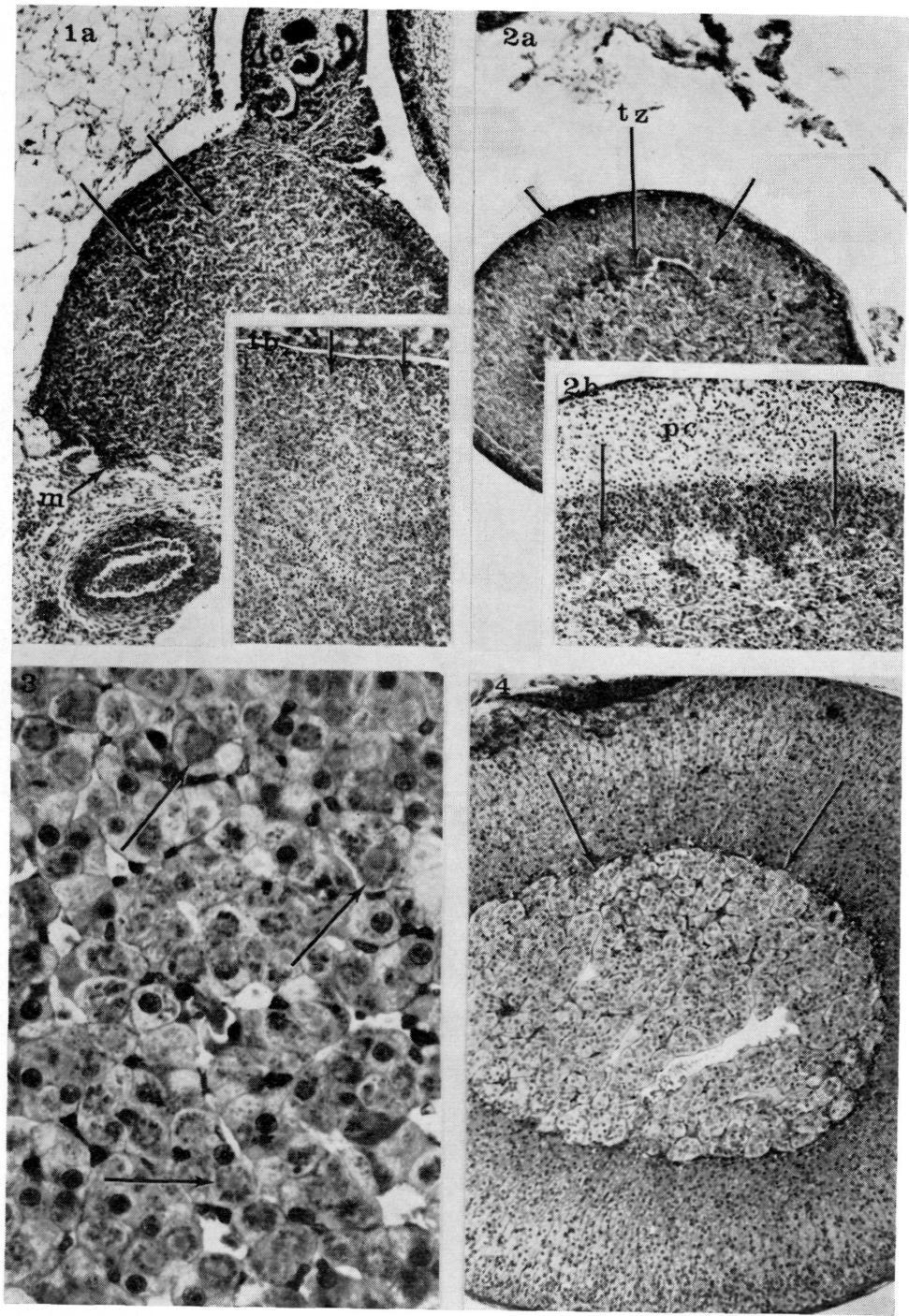
DISCUSSION

The transient-zone (Sucheston and Cannon, 1968a) is an integral portion of the developing adrenal cortex in man and the mouse, but the present study questions the morphological and functional homologies attributed to this zone (Blackman, Jr., 1946; Howard, 1930, 1939; Nicander, 1952–1953; Velican, 1948).

In man the transient-zone is the first region of the adrenal gland to develop; this occurs during the fourth week of gestation (Stage 1), while the permanent cortex forms secondarily at eight and one-half weeks of gestation (Stage 2). In the mouse the permanent cortex has formed by the time of parturition (Stage 1) and the transient-zone does not appear until the eighth postnatal day (Stage 1). The disappearance of the transient-zone in the human male and female occurs from approximately 28 weeks of gestation to one year postnatal (Stage 4). In the mouse, the onset of puberty in the male is simultaneous with the disappearance of the transient-zone (Stage 4), while in contrast, the transient-zone of the female mouse is lost during the first pregnancy. Thus, it appears that the functional

EXPLANATION OF PLATE

- FIGURE 1a. Human male at four weeks gestation. Entire adrenal gland is composed of the transient-zone (arrows). Medullary material (m) on medial aspect of the cortical area in the dorsal mesentery. Azan, $\times 100$.
- FIGURE 1b. Human male at eight and one-half weeks gestation. Secondary proliferation of coelomic epithelium forms permanent cortex (arrows) around transient-zone. Azan, $\times 100$.
- FIGURE 2a. Male mouse ten days postnatal. The permanent cortex is present at birth (arrows). The transient-zone appears as juxtamedullary clusters of cells (tz) by the second postnatal week. Azan, $\times 100$.
- FIGURE 2b. Female mouse twenty days postnatal. Permanent cortex (pc) and deeply acidophilic transient-zone cells (arrows) compose the adrenal cortex. Azan, $\times 100$.
- FIGURE 3. Transient-zone exhibits juxtannuclear PAS-positive coarse granules surrounding a homogeneous cytoplasmic mass (arrows) in the eighteen week-old human male fetal adrenal gland. PAS, $\times 450$.
- FIGURE 4. Male mouse at thirty-six days postnatal. A permanent inner connective tissue capsule (arrows) separates the cortex from the medulla. Azan, $\times 100$.



activity of the transient-zone is closely correlated with sexual maturity in the mouse. In man no such correlation is observed, and it is doubtful if the transient-zone is identical in function in man and the mouse as previously stated (Benua and Howard, 1950; Howard-Miller, 1927-1928).

Previous observations have shown that, prior to the initiation of mitotic activity

TABLE 1
Stages in transient-zone development

Stage	Man	Mouse
1	4-8 weeks gestation (5 specimens). Primary proliferation of coelomic epithelium forms the transient-zone. ¹ These cells are large, polyhedral with granular basophilic cytoplasm and <i>indistinct</i> cell boundaries.	8-16 days postnatal (36 specimens). <i>Permanent cortex</i> ² present at birth. Transient-zone appears gradually as juxtamedullary scattered clusters of cells. These cells are small, compact with deeply <i>acidophilic</i> cytoplasm and <i>indistinct</i> cell boundaries.
2	8.5-17 weeks gestation (4 specimens). Secondary proliferation of coelomic epithelium forms a cap over the transient-zone; this is the <i>permanent cortex</i> . Transient-zone cells are large, polyhedral, with granular <i>acidophilic</i> cytoplasm and <i>distinct</i> cell boundaries.	Stage 2 is not comparable in man and the mouse.
3	18-27 weeks gestation (7 specimens). Transient-zone occupies approximately <i>three-fourths</i> of entire adrenal gland. Juxtannuclear PAS+coarse granules surrounding a homogeneous cytoplasmic mass are present at beginning of Stage 3 and decrease gradually toward end of this stage. These cells also are lipid +, cholesterol ? ³ .	17-28 days postnatal (48 specimens). Transient-zone occupies approximately <i>one-third</i> of entire adrenal gland. These cells are PAS-, lipid-, cholesterol-, but demonstrate light acidophilia adjacent to zona fasciculata with gradation to deep acidophilia adjacent to medulla.
4	28 weeks gestation through 6 months-1 year postnatal (20 specimens). Disappearance of majority of transient-zone cells in <i>male</i> and <i>female</i> by loss of PAS+granules, cytoplasmic acidophilia, lipid and cholesterol. At end of Stage 4, a few cells remain which retain characteristics of transient-zone. At 36 weeks postnatal a connective tissue stroma, present for approximately one week, forms <i>inner capsule</i> separating cortex from medulla.	29-45 days postnatal (28 specimens). Disappearance of majority of transient-zone cells; <i>male</i> (approximately 45 days); <i>female</i> (first pregnancy); by loss of cytoplasmic acidophilia. A <i>permanent inner</i> connective-tissue capsule separates cortex from medulla.
5	2 years-puberty (11-15 years) (14 specimens). Establishment and stabilization of definitive ⁴ zonal patterns in male and female by: 1) proliferation of permanent cortex; 2) maturation of transient-zone; 3) growth of medulla. No <i>remnant</i> of transient-zone cells apparent.	46-52 days postnatal (12 specimens). Establishment and stabilization of definitive zonal patterns by what appears to be the same processes as those occurring in man. <i>Remnants</i> of transient-zone may be represented by narrow irregular band of cells adjacent to inner connective-tissue capsule.

¹Area of cortex that has not yet appeared morphologically to have delineated into the innermost portion of zona fasciculata and the entire zona reticularis.

²Area of cortex that gives rise to zona glomerulosa and the outermost portion of the zona fasciculata.

³Inconclusive.

⁴Stage at which three zones (i.e., zona glomerulosa, zona fasciculata, zona reticularis) of cortex are clearly defined with no apparent remnant of transient-zone.

within the permanent cortex, giving rise to the three zones of the adrenal cortex (i.e., zona glomerulosa, zona fasciculata, zona reticularis) in man and the mouse, involution of the transient-zone takes place as a result of hemorrhage and cell loss (Benner, 1940; Howard-Miller, 1927-1928; Lanman, 1961; McNeil, 1947; Velican, 1948; Waring, 1935-1936). However, the authors' earlier study (1968b) revealed that the permanent cortex in man gives rise to the zona glomerulosa and outermost portion of the zona fasciculata while the maturation of the transient-zone slowly establishes the innermost portion of the zona fasciculata and the entire zona reticularis. The present investigation also shows that the permanent cortex and the transient-zone in the mouse give rise to the same zones of the adrenal cortex as in man.

At four weeks of gestation (Stage 1), the transient-zone in man is composed of large, polyhedral cells, possessing granular basophilic cytoplasm and indistinct cell boundaries. At eight and one-half weeks of gestation (Stage 2), as the permanent cortex is forming, the cells of the transient-zone become acidophilic and possess distinct cell boundaries. In the mouse the transient-zone always consists of small, compact cells with deeply acidophilic and indistinct cell boundaries.

The most striking phase in the development of the adrenal gland in man is observed in the transient-zone of 18-week-old fetuses (Stage 3), with the appearance of juxtanuclear PAS-positive coarse granules surrounding a homogeneous cytoplasmic mass. This juxtanuclear material is specific for the cells of the transient-zone and begins to decrease gradually at the time of birth and a few months thereafter. The location of the homogeneous mass may identify the Golgi region, an area of high metabolic activity within the differentiating transient-zone (Sucheston and Cannon, 1968b). The Golgi region has been described previously in the cells of the cortical zones, and an increase in the size of this region appears to occur with increased functional activity (Johannisson, 1968; Lever, 1955). The transient-zone of the mouse does not demonstrate the presence of juxtanuclear PAS-positive material surrounding a homogeneous cytoplasmic mass, although its cytoplasm continually demonstrates acidophilia.

A positive but slight reaction for lipid and a questionable reaction for the presence of cholesterol was seen in the transient-zone of man in the present study. In the mouse a positive reaction for lipid and cholesterol was never observed, thus verifying earlier findings (Benua and Howard, 1950; Jones, 1952). Although a negative or inconclusive result with regard to the presence of lipid and cholesterol was observed, this may indicate only that the concentration of these steroid hormone precursors is not sufficient to be detected by the histochemical methods employed. Nevertheless, distinct differences do exist between man and the mouse.

The presence of PAS-positive material within the cells of the transient-zone is a normal phase in the maturation of the adrenal cortex in man. Although this particular facet in adrenal development has not been pursued, there have been references to PAS-positive giant epithelial cells within the human transient-zone, and in the adrenals of children with adrenocortical carcinoma (Craig and Landing, 1952; Kampmeier, 1927-1928; Kúnski, 1959; Lowenthal, Leszynsky, Marcus and Zondek, 1957-1958; Nobuaki, 1961). In the mouse, remnants of the transient-zone may be represented by a narrow irregular band of cells adjacent to the inner connective tissue capsule. Thus, contrary to previous investigations, the present study demonstrates that the transient-zone of both man and the mouse is a phase in the maturation of the adrenal cortex, but indicates that there is a difference in its morphological development and probable functional activity in these two mammalian species.

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